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ANTIPARASITIC ACTIVITY OF BLUE-GREEN ALGAE EXTRACT

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SUMMARY PAGE

F&E

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Summary Statement: Extracts from 10 species of Cyanophyta were applied separately to axenic cultures of Trichomonas hominis, T. muris, and T. augusta. During an incubation period of 7 days, extracts from the 10 algae inhibited the growth of two or more of the protozoan parasites at the interval corresponding to the maximum growth phase of the controls. Lyngbya sp. extract at a concentration of 0.45 ml/ml medium was most effective, with 98 per cent inhibition in T. hominis and 100 per cent in T. muris. Extracts from the two species of Anabaena were least effective.

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ANTIPARASITIC ACTIVITY OF BLUE-GREEN ALGAE EXTRACT

By

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WACO, TEXAS

Abstract

Extracts from 10 species of Cyanophyta were applied separately to axenic cultures of Trichomonas hominis, T. muris, and T. augusta. During an incubation period of 7 days, extracts from the 10 algae inhibited the growth of two or more of the protozoan parasites at the interval corresponding to the maximum growth phase of the controls. Lyngbya sp. extract at a concentration of 0.45 ml/ml medium was most effective, with 98 per cent inhibition in T. hominis and 100 per cent in T. muris. Extracts from the two species of anabaena were least effective.

Introduction

Since the earliest recorded occurrence of a toxic waterbloom (Francis, 1878), more than fifty outbreaks of algae poisoning in wild and domestic animals and in humans have been reported. In a previous report (Davidson, 1959) of algae poisoning, in wild and domestic animals, involving a waterbloom of Nostoc rivulare, a review of all available data concerning the toxic properties of the Cyanophyta was included. Lefevre (1952), Wurtz (1949),

Goryuvona (1955), and Davidson (1961, 1962) obtained substances from certain species of Cyanophyta which exhibit an antibacterial activity against certain bacteria and other microorganisms. Based on data obtained from laboratory tests concerning the antibacterial activities of certain Cyanophyta, Flint (1946) suggests this group of algae as a possible source of additon antibiotics. Davidson (1959) obtained substances from this group of algae which inhibit pigment formation in Serratia marscescens, a character exhibited by many antibiotics. Little or no data is available, however, concerning the antiparasitic action of the blue-green algae or their exudates. In the present study, crude extracts obtained from these algae were tested for their possible antiparasitic action against Trichomonas hominis, T. augusta, and T. muris.

Materials and Methods

Algae Strains. The 15 species of algae used in this investigation were obtained from the Indiana University Stock Culture Collection, Bloomington, Indiana: Oscillatoria tenuis, O. formosa, Nostoc calcicola, N. ellipsosporum, N. punctiform, Anabaena catenula, A. variabilis, Symploca muscorum, Phormidium flaveolarum, Tolypothrix distorta, Calothrix membranacea, Fremyella diplosiphon, Cylindrosperum sp., Lyngbya sp, Plectonema boryanum.. A bacteria-free culture of each species was established and maintained in modified Benecke's liquid medium and on Benecke's medium with 1.5 per cent added agar (De, 1939). The stock culture room was provided with a constant temperature of 20-24° C., light intensity of 50-75 ft-c, and relative humidity of 50-60 per cent.

In order to provide an adequate and reliable supply of algal material, bacteria-free sub-cultures were established in 2 liter Fernbach flasks containing 1 liter modified Benecke's or modified Chu's medium (Gerloff, et.al., 1960). The cultures were aerated with a continuous stream of air, to which 0.5 per cent carbon dioxide was added 4 hours daily. Constant illumination (500 ft-c) was provided by 40W cool-white fluorescent tubes, and the room temperature was thermostatically controlled at 27° C. (±2°).

Preparation of algal extract. The method previously reported (Davidson, 1961, 1962), for the preparation of an aqueous extract containing an antibacterial factor was modified for this study. The algal cells were harvested by centrifugation and washed 3 times by centrifugation from sterile distilled water. The algal mass was blotted, weighed, and ground in a tissue grinder with 30 ml. sterile distilled water. The suspension was added to the transducer of a 10 KC Raytheon sonic oscillator and exposed to a sound field of approximately 50 acoustical watts (1.2-1.3 amp audio frequency current) for 30 minutes. The temperature of the suspension was maintained at 4-6° C. during the treatment. Microscopic examination showed complete disruption of the algae cells. The cell walls were removed by centrifugation at 2000 X G for 10 minutes. The supernatant, which contained the water-soluble fraction of the cytoplasm and matrix, was sterilized by passing it through a sintered-glass bacterial filter (porosity 0.9-1.0u). Tests for the presence of pigments and for bacteria in the extract were negative.

Trichomonid cultures. Three species of trichomonas were employed: Trichomonas hominis Davaine, T. muris Grassi, and

T. augusta Alexeieff. The culture of T. hominis in Modified Tsuchiyas' Endamoeba Histolytica Medium (Craig, 1948) was obtained from the State Department of Public Health, Austin, Texas, where it was taken directly from an infected patient. Sub-cultures were established in 10 ml. Drbohlav's Modified Ringer's Solution (Craig, 1948) to which were added 20 mg. Loeffler's Blood Serum and incubated at a temperature of 32° C. Sub-culturing was necessary every 2-3 days. Bacteria-free cultures were obtained by continuous sub-culturing in Drbohlav's Modified Ringer's containing 1.9 mg. penicillin G Potassium and 1 mg. streptomycin sulphate per ml. culture medium. Tests for the presence of bacteria were negative.

Trichomonas muris was isolated from the colon of a white rat. Axenic cultures were obtained by the use of antibiotics in Drbohlav's Modified Ringer's Solution as for T. hominis.

The original culture of Trichomonas augusta was obtained from the rectum of Hyla Cinerea, the common green tree-frog. The content of the rectum was placed in 100 ml. Ringer's Solution (for cold-blooded vertebrates), to which were added 200 mg. Loeffler's Blood Serum and 100 mg. gastric mucin (Craig, 1948). This was separated into 20 ml. alequotes in 30 ml. bacterial test tubes. The frog rectum was cut into 5 sections, one of which was introduced into each tube. The tubes were maintained at 27° C. Previous reports indicate that cultures of T. augusta had been maintained as long as 365 days. In this study, however, the original cultures remained healthy for more than two years. The original level of the solution in each tube was maintained by the addition of distilled water, but no additional nutrients

were required. Bacteria-free cultures were established as for T. hominis, using one-half strength Drbohlav's Modified Ringer's for the culture medium.

Testing the extract. Trichomonas hominis, T. muris. To each of 6 standard bacteriological test tubes 5 ml. sterile double strength Drbohlav's Modified Ringer's Solution was added. To each of 5 of these tubes was added one of the following: 1, 2, 3, 4, 5 ml. of the algal extract to be tested. Sterile distilled water was added to the first 4 tubes until the solution level was the same throughout the 5 tubes (10 ml.). The extract concentrations in the tubes therefore were 0.09, 0.18, 0.27, 0.36, and 0.45, ml/ml medium, respectively. The sixth tube was prepared by adding 5 ml. sterile distilled water, and was used as the control.

Two 4-day bacteria-free cultures of T. hominis were thoroughly mixed and the number of organisms per ml. medium was determined. Two ml. of the culture was added to each of the 5 extract-treated tubes and to the control. The number of organisms per ml. medium in each of the six tubes was identical. The six tubes were incubated at 32° C. The number of organisms per ml. culture medium in each tube was determined daily with the aid of a hemacytometer, an improved Neubauer counting chamber, and a compound microscope. Each of the data represents the average of five separate counts of organisms from a single tube.

Trichomonas augusta. Since maximum growth of T. augusta occurred in one-half strength Drbohlav's Modified Ringer's Solution with added nutrients, this medium was employed throughout the series of tests.

Into each of 6 tubes were placed 5 ml. Drbohlav's Modified Ringer's Solution (single strength), 20 mg. Loeffler's Blood Serum, and 20 mg. gastric mucin. Algal extract and distilled water were added as in the T. hominis tests. Each tube was inoculated with 2 ml. bacteria-free medium containing a measured number of T. augusta. The 6 tubes were incubated at a temperature of 21° C. (±2°). Counts were made at 24 hour intervals as in previous tests.

Aseptic techniques were employed in all tests with the three trichomonads.

Results

The effect of blue-green algae extracts on the growth of Trichomonas hominis, T. muris, and T. augusta during a period of 7 days is shown in Tables 1, 2, and 3. The data X 1000 represent the number of organisms per ml. medium in the 5 extract-treated cultures and in the control at 1-day intervals during the incubation period. The number of organisms per ml. medium was identical in the 6 tubes at the beginning of each experiment. Each of the data represents the average of five separate counts of organisms from a single tube.

In general the 5 concentrations of extract stimulate the growth of the 3 trichomonads during the first 2-3 days of growth. In some cases growth was stimulated throughout the growth period (fig. 16), while in others growth was stimulated during the first 2-3 days followed by a sudden inhibitory effect.

Maximum growth in control cultures occurred at the third or

fourth day of the growth period. Data taken on extract-treated cultures at intervals corresponding to the maximum growth phase of the control, indicate that each of the 10 extracts exhibited some antiparasitic activity against at least two of the trichomonads. Extracts from Lyngbya sp. were most effective, with 98 per cent inhibition in T. hominis cultures and 100 per cent in T. muris. At this period extracts from the two species of Anabaena were least effective.

The greatest amount of inhibition of growth in extract-treated cultures did not occur at the maximum growth phase of the controls in every instance. In some, maximum inhibition occurred before and others after the maximum growth phase in the controls was reached.

Summary

Each of the extracts from the 10 species of Cyanophyta showed some antiparasitic activity against at least two of the trichomonads tested. Extracts from 5 species inhibited the growth of all three trichomonads. In extracts which exhibited antiparasitic activity, the per cent inhibition varied directly with the concentration of the extract.

Many of the extracts stimulated growth of the trichomonads, as high as 250 per cent in Nostoc ellipsosporum extract. Some stimulated growth during the early phases of the growth period and inhibited growth later. In other cases the antiparasitic activity occurred during the early phases.

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Table 1. Effect of blue-green algae extract on the growth of Trichomonas hominis, T. muris, and T. augusta during an incubation period of 7 days. The data X 10^3 represent the number of organisms per ml. medium in extract-treated cultures and in the controls at 1-day intervals during the incubation period.

Algae	Ml. Ext./ Ml. Med.	Trichomonas hominis							Trichomonas muris							Trichomonas augusta						
		Growth Period in Days							Growth Period in Days							Growth Period in Days						
		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Oscillatoria formosa	.09	270	590	580	740	870	510	380	160	250	400	540	630	410	320	19	45	105	115	143	153	210
	.18	280	640	670	790	440	510	200	160	210	370	470	520	430	290	15	30	78	96	148	160	210
	.27	340	660	750	740	610	620	190	210	290	370	540	510	520	150	11.6	25	60	133	135	106	215
	.36	290	650	840	720	640	490	230	130	190	540	490	450	380	140	23	23	81	178	195	183	160
	.45	270	430	870	690	720	520	270	150	280	510	400	460	490	180	20	18	66	126	160	192	200
	Control	280	470	770	810	700	620	270	110	160	290	290	430	520	420	23	25	81	90	180	138	188
Tolypothrix distorta	.09	103	350	480	552	720	542	377	25	215	152	280	242	377	172	40	127	167	277	190	212	190
	.18	62	452	480	527	527	515	292	25	120	277	187	347	367	457	37	42	165	295	217	255	217
	.27	37	352	515	275	407	560	547	25	100	187	245	222	250	232	47	37	75	257	412	295	412
	.36	67	155	472	677	312	397	720	20	187	177	182	217	320	297	30	32	57	332	340	375	340
	.45	70	162	535	590	297	520	335	30	122	170	267	262	370	260	27	10	40	112	337	332	337
	Control	61	325	535	380	345	350	367	17	80	135	97	112	177	237	55	125	120	95	180	170	180
Anabaena catenula	.09	230	500	500	570	390	200	110	200	340	330	440	230	220	50	30	20	20	40	80	80	150
	.18	350	470	440	580	650	230	180	160	280	380	480	340	190	116	10	10	0	20	20	50	100
	.27	160	450	440	470	620	480	400	160	260	420	390	300	160	80	20	10	20	20	30	70	100
	.36	220	390	460	430	490	420	210	240	280	410	360	290	190	40	10	10	10	10	30	90	140
	.45	230	410	510	410	530	380	220	140	270	380	260	320	310	80	10	20	10	10	30	90	110
	Control	240	270	410	440	530	320	230	180	240	250	125	230	230	110	30	40	40	70	70	90	130
Nostoc calcicola	.09	120	357	352	555	435	282	250	25	85	90	130	145	95	100	10	12	17	37	47	65	47
	.18	2	307	482	485	437	327	237	17	50	115	62	152	102	135	5	10	10	10	130	72	130
	.27	90	305	322	560	407	465	157	10	45	55	67	102	105	77	2.5	10	10	17	112	70	112
	.36	112	112	422	525	680	240	132	10	22	80	122	70	87	85	2.5	10	10	25	125	55	125
	.45	132	305	327	410	258	237	122	10	30	90	100	157	150	122	2.5	10	10	25	127	65	127
	Control	110	425	290	320	312	197	132	32	70	87	100	117	142	110	10	10	20	57	180	197	180

Table 2. Effect of different concentrations of blue-green algae extract on the growth of Trichomonas hominis, T. muris, T. augusta during an incubation period of 7 days. The data x1000 represent the number of organisms per ml. medium in extract-treated cultures and in the controls at 1-day intervals during the incubation period.

Algae	Ml Ext/ Ml Med.	Trichomonas hominis							Trichomonas muris							Trichomonas augusta						
		Growth Period in Days							Growth Period in Days							Growth Period in Days						
		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Calothrix membranacea	.09	60	270	500	460	360	300	170	127	200	380	525	245	160	92	110	130	280	500	650	1220	850
	.18	130	380	550	620	410	180	90	97	282	215	302	222	200	147	70	160	310	620	950	1150	800
	.27	170	440	460	570	260	70	30	80	190	307	390	215	110	132	50	230	330	530	900	1100	900
	.36	170	710	750	610	140	50	30	62	185	280	222	350	217	185	50	160	280	550	980	1200	930
	.45	180	420	820	780	90	30	10	45	165	205	340	297	242	180	30	160	180	370	530	1220	1270
	Control	110	320	580	550	250	180	110	92	147	222	352	415	217	123	40	120	400	590	650	1080	1220
Symploca muscorum	.09	110	300	410	176	370	300	298	120	257	360	254	627	490	112	50	90	146	254	270	180	233
	.18	230	405	306	340	330	211	260	80	270	250	318	513	250	210	10	50	163	202	145	270	270
	.27	70	120	304	520	480	348	328	90	320	398	526	454	177	136	10	40	180	213	211	270	360
	.36	180	302	216	260	340	315	258	50	316	258	216	485	360	325	40	100	213	263	290	286	205
	.45	230	420	940	615	370	470	286	150	310	520	478	370	223	80	20	122	318	180	310	343	228
	Control	360	290	530	610	423	418	270	70	170	196	416	536	193	230	10	60	146	133	210	186	190
Lyngbya sp.	.09	140	475	470	477	540	210	272	56	81	136	215	220	150	176	13	18	85	210	500	430	200
	.18	105	440	567	482	462	400	495	12	36	44	210	275	120	232	24	58	52	127	325	352	380
	.27	66	95	220	250	420	485	352	22	18	27	15	5	5	2.5	27	31	64	90	260	320	425
	.36	55	80	242	327	457	690	252	33	55	38	10	1.6	0	0	10	18	20	143	390	535	692
	.45	55	12	10	10	0	0	0	28	20	0	0	0	0	0	18	26	38	100	240	470	720
	Control	108	400	620	430	417	267	232	33	63	93	110	140	115	102	20	32	45	50	116	87	63
Anabaena variabilis	.09	180	350	420	460	590	300	240	130	260	290	440	360	390	340							
	.18	170	400	790	530	470	340	190	190	270	410	340	450	390	310							
	.27	180	430	570	620	590	490	180	160	230	320	420	550	440	270							
	.36	150	410	660	480	470	290	130	140	250	280	560	460	360	270							
	.45	160	370	510	460	520	560	210	140	250	370	480	440	460	290							
	Control	60	380	390	510	430	390	330	130	180	290	360	380	320	270							

Table 3. Effect of different concentrations of blue-green algae extract on the growth of Trichomonas hominis, T. muris, and T. augusta during an incubation period of 7 days. The data x 1000 represent the number of organisms per ml. medium in extract-treated cultures and in the controls at 1-day intervals during the incubation period.

Algae	Ml. Ext/ Ml. Med.	Trichomonas hominis							Trichomonas muris							Trichomonas augusta						
		Growth Period in Days							Growth Period in Days							Growth Period in Days						
		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Nostoc ellipsosporum	.09	70	120	140	190	310	240	260														
	.18	50	110	180	200	390	300	140														
	.27	30	130	180	300	300	230	130														
	.36	20	120	240	350	290	130	120														
	.45	10	80	240	280	330	220	120														
	Control	20	180	270	300	320	340	100														
Phormidium fâveolarum	.09								100	307	385	522	422	365	162	17	25	52	120	182	287	135
	.18								75	205	240	317	432	370	85	12	10	37	55	127	240	345
	.27								87	152	285	452	505	280	340	10	10	17	67	125	220	285
	.36								70	155	225	552	460	332	312	10	7.5	17	27	85	212	215
	.45								90	165	340	275	630	252	185	10	5	17	27	87	305	285
	Control								107	155	217	335	350	200	100	20	20	40	92	150	237	165
	.09																					
	.18																					
	.27																					
	.36																					
	.45																					
	Control																					
	.09																					
	.18																					
	.27																					
	.36																					
	.45																					
	Control																					

Table 4. Effect of different concentrations of blue-green algae extracts on the growth of Trichomonas hominis, T. muris, and T. augusta during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. in the controls at the maximum growth phase of the controls. Negative values represent stimulation of growth.

Source of Extract	Trichomonas hominis					Trichomonas muris					Trichomonas augusta				
	Ml. ext./ml. med.					Ml. ext./ml. med.					Ml. ext./ml. med.				
	.09	.18	.27	.36	.45	.09	.18	.27	.36	.45	.09	.18	.37	.36	.45
Calothrix membranacea	14	5	21	-29	-41	41	47	48	17	28	30	34	26	24	-4
Nostoc ellipsosporum	29	12	32	62	35										
Oscillatoria formosa	9	3	9	11	15	21	17		27	6	21	18	25	-8	11
Anabaena variabilis	10	-4	-22	6	10	5	-18	-45	-21	-16					
Anabaena catenula	26	-23	17	8		-69	-85	-50	-38	-42	-15	23	23	-8	35
Lyngbya sp.	25	8	64	60	98	-57	-97	97	99	100	-330	-180	-129	-150	-107
Tolypothrix distorta	10	10	4	12		-12	-105	-38	-31	-26	-2	66	70	74	92
Nostoc calcicola	16	27	28	20	28	33	28	26	37	-5	65	63	61	72	65
Phormidium faveolarum						-21	-23	-44	-31	-80	-21	-1	72	10	-29

Reference Key To Curves Plotted in Figs. 1-22

Ml. Extract/Ml. Medium

.09.....

.18— — — —

.27—·—·—·

.36———— —

.45————

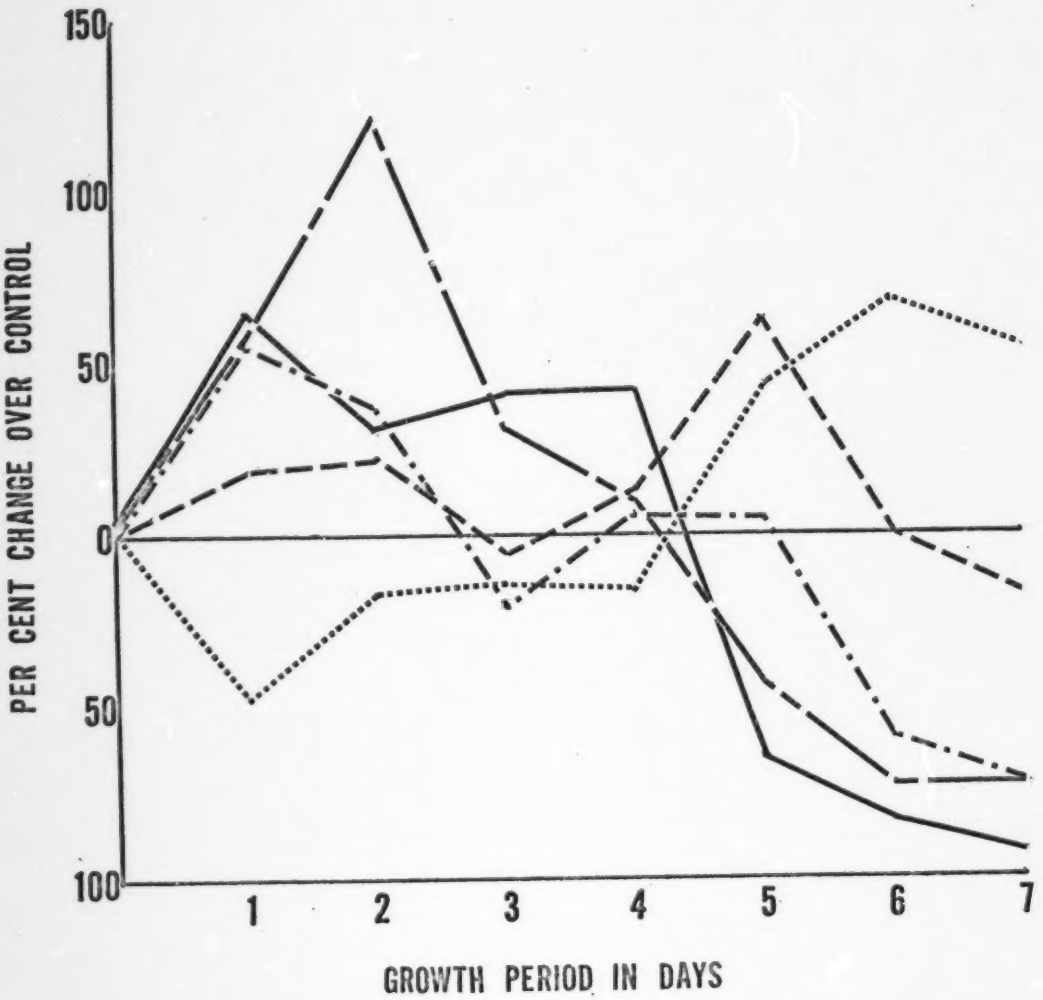

Fig. 1. Effect of different concentrations of Calothrix membranacea extract on the growth of Trichomonas hominis during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

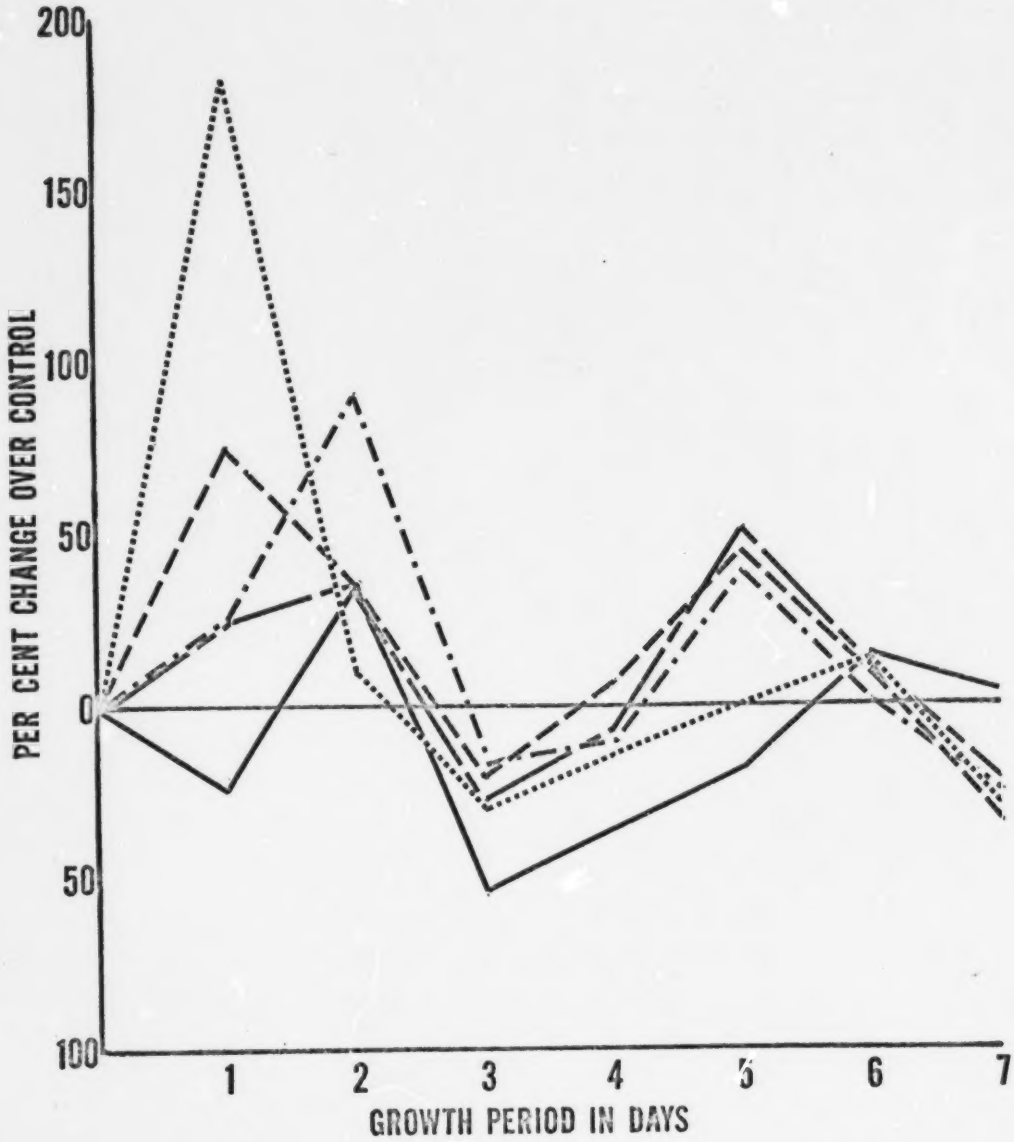

Fig. 2. Effect of different concentrations of Calothrix membranacea extract on the growth of Trichomonas augusta during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

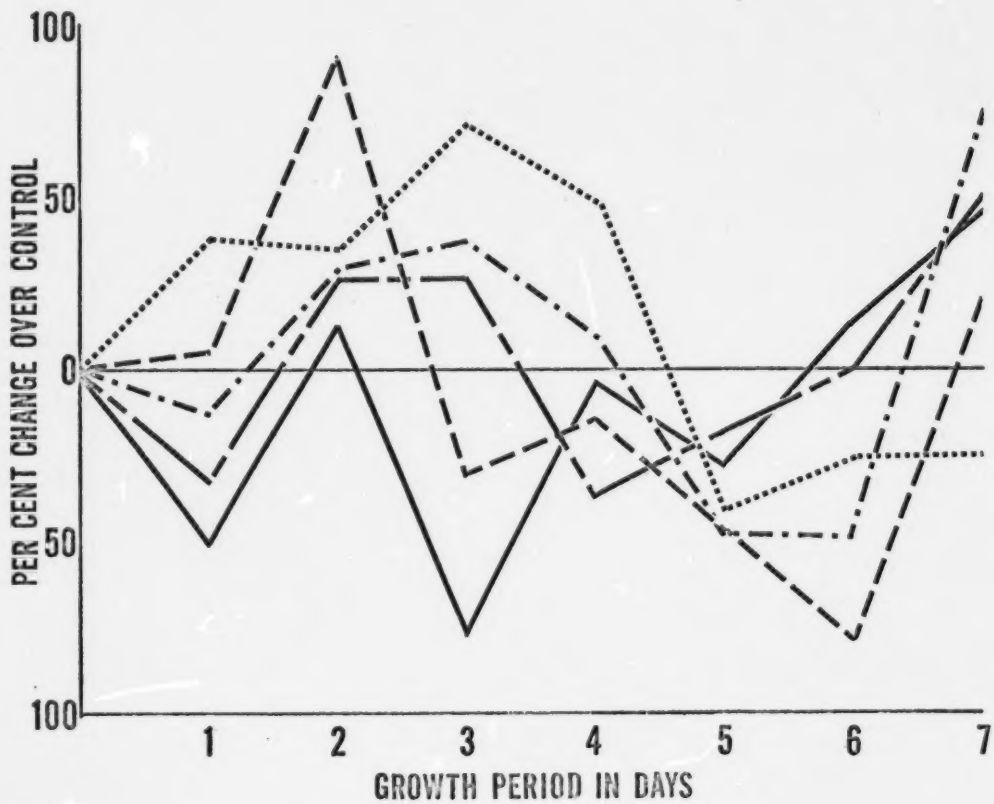

Fig. 3. Effect of different concentrations of Calothrix membranacea extract on the growth of Trichomonas muris during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

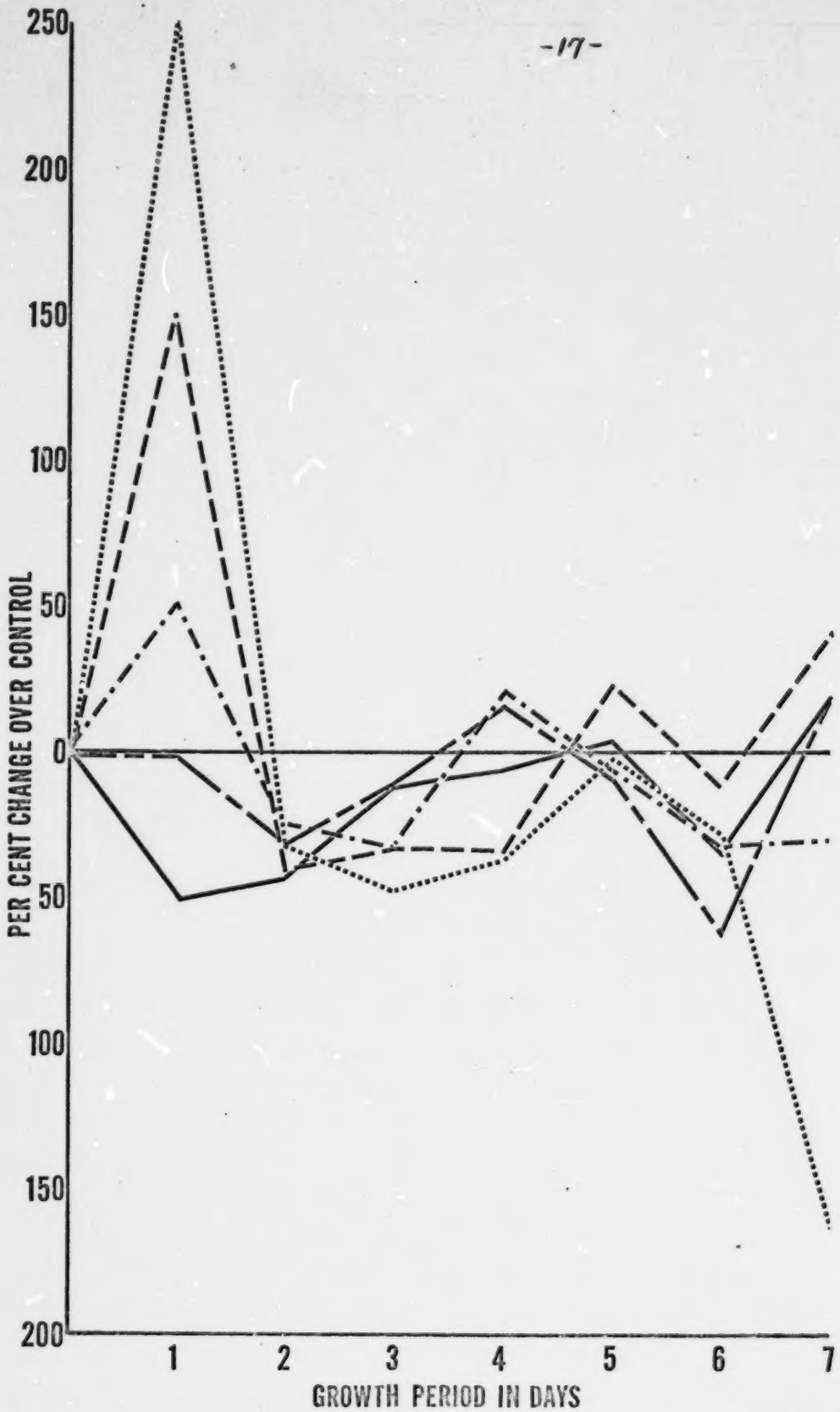

Fig. 4. Effect of different concentrations of Nostoc ellipsosporum extract on the growth of Trichomonas hominis during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. in the control.

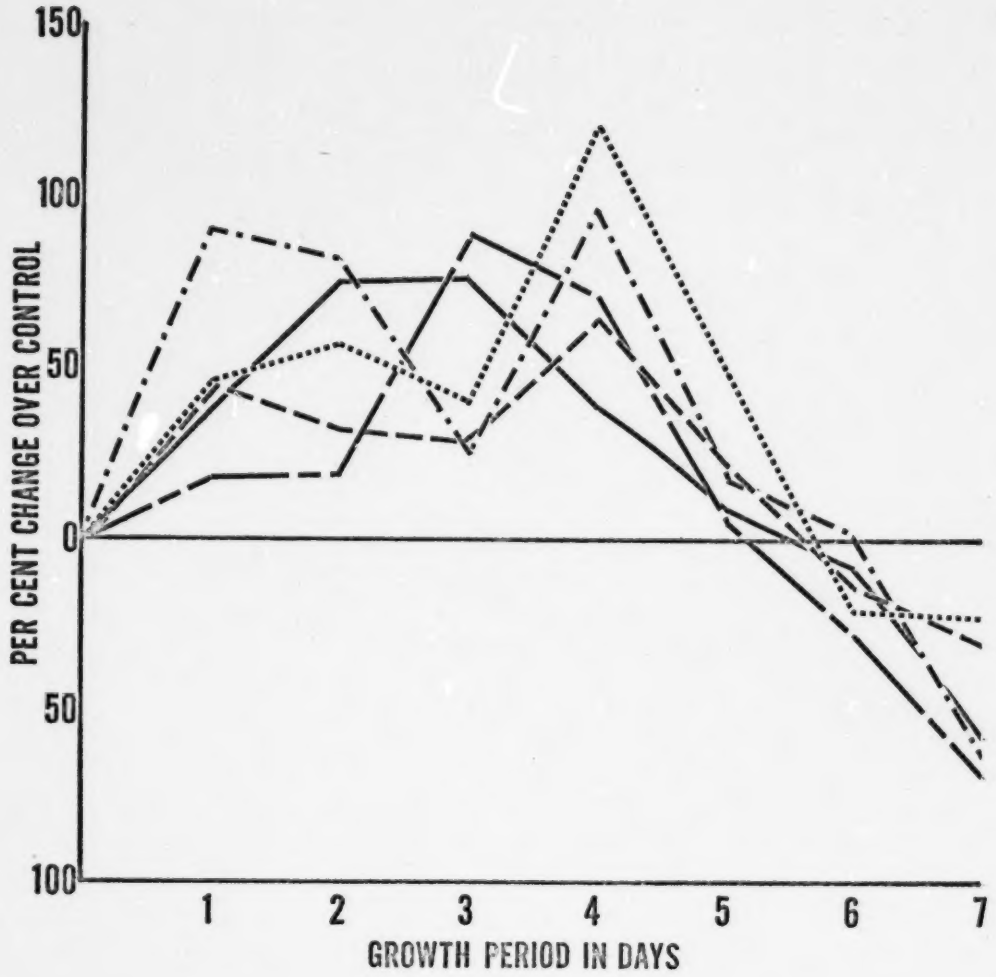

Fig. 5. Effect of different concentrations of Oscillatoria formosa extract on the growth of Trichomonas muris during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

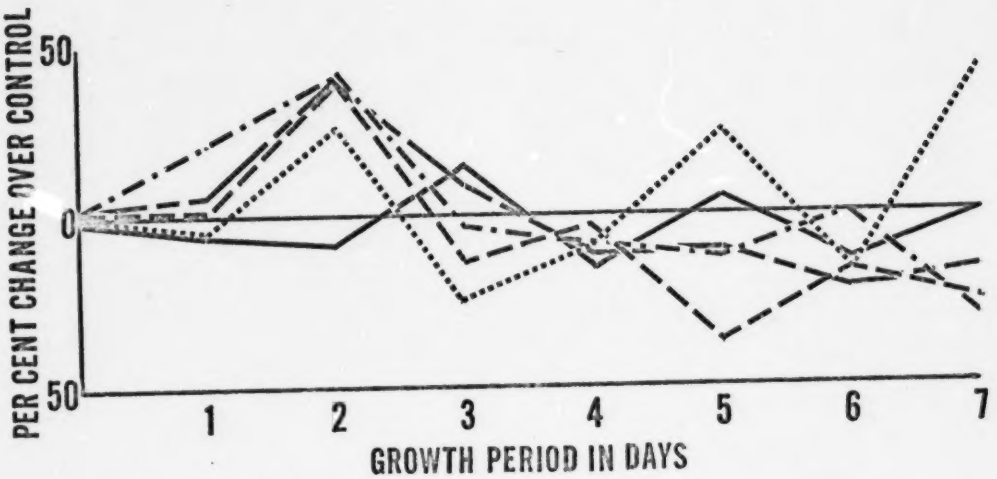

Fig. 6. Effect of different concentrations of Oscillatoria formosa extract on the growth of Trichomonas hominis during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

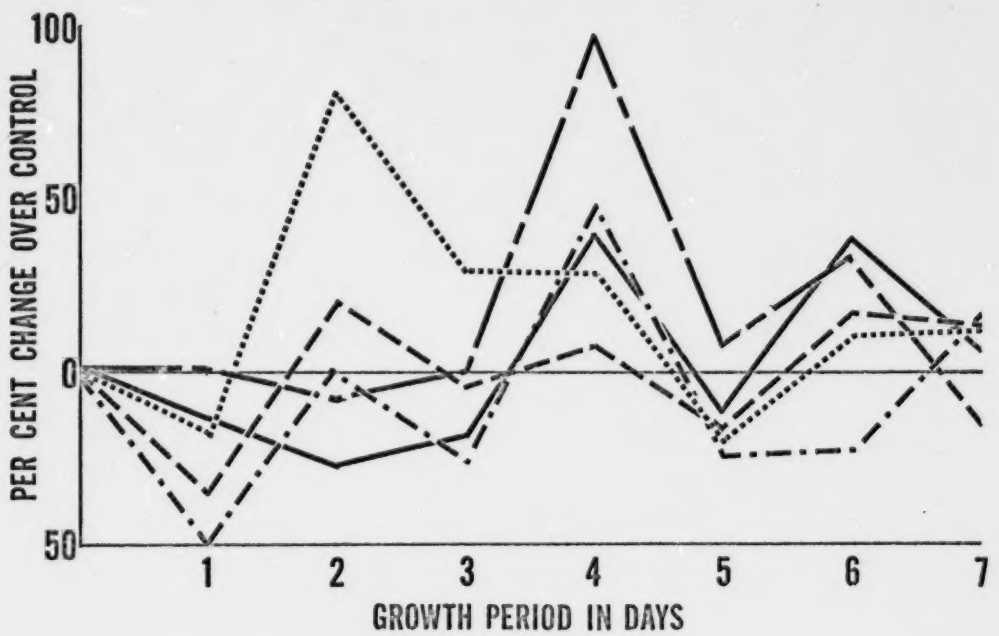

Fig. 7. Effect of different concentrations of Oscillatoria formosa extract on the growth of Trichomonas augusta during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

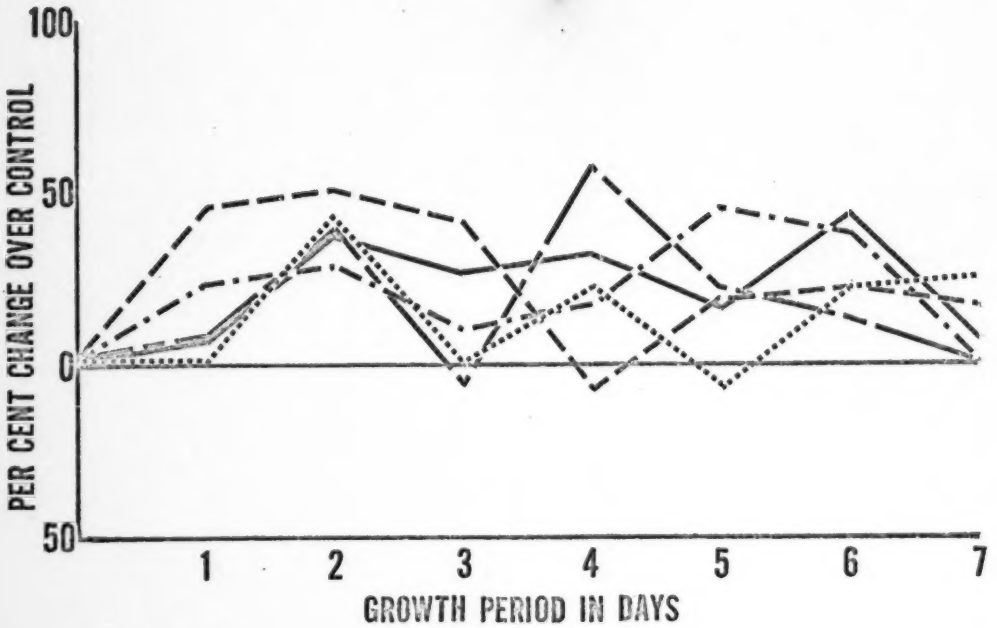

Fig. 8. Effect of different concentrations of Anabaena variabilis extract on the growth of Trichomonas muris during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

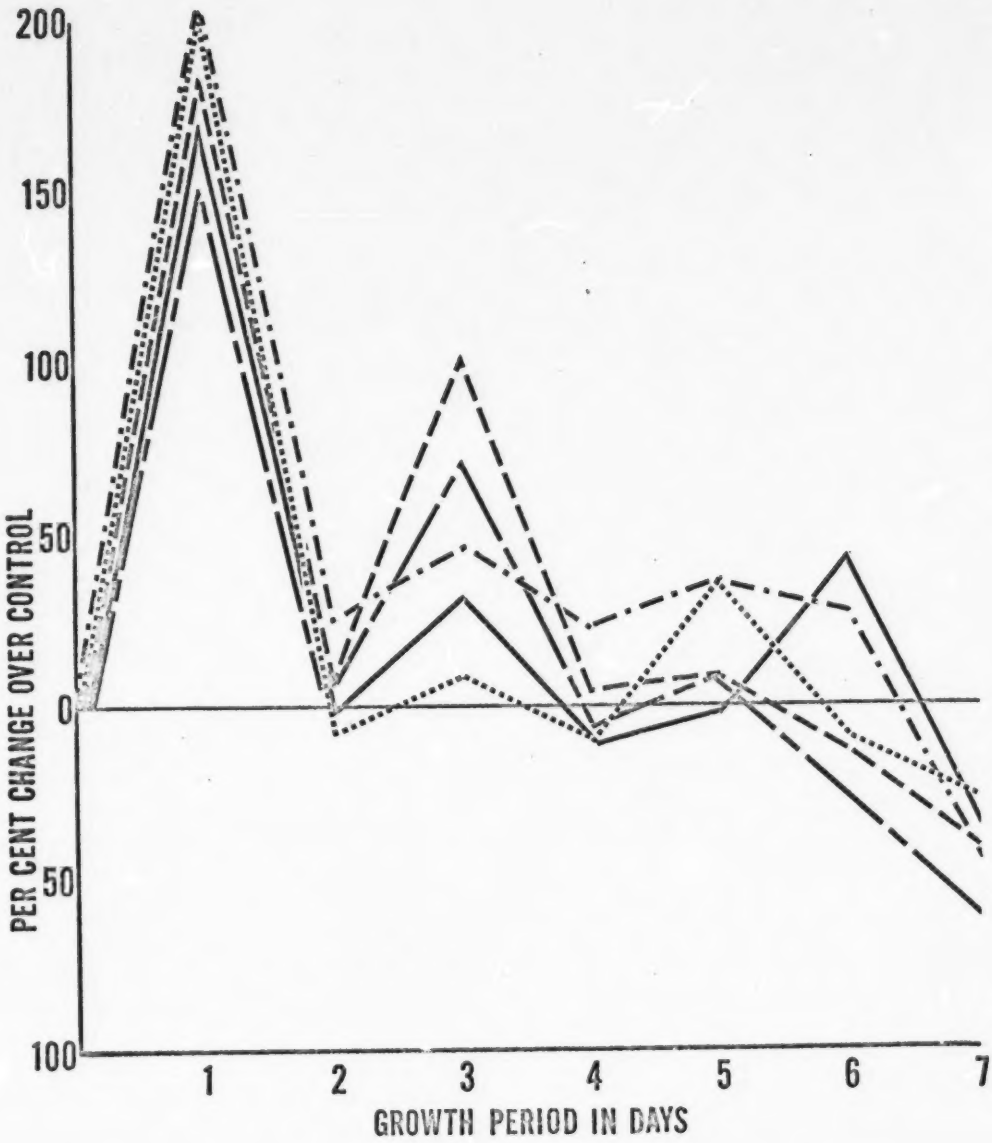

Fig. 9. Effect of different concentrations of Anabaena variabilis extract on the growth of Trichomonas hominis during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

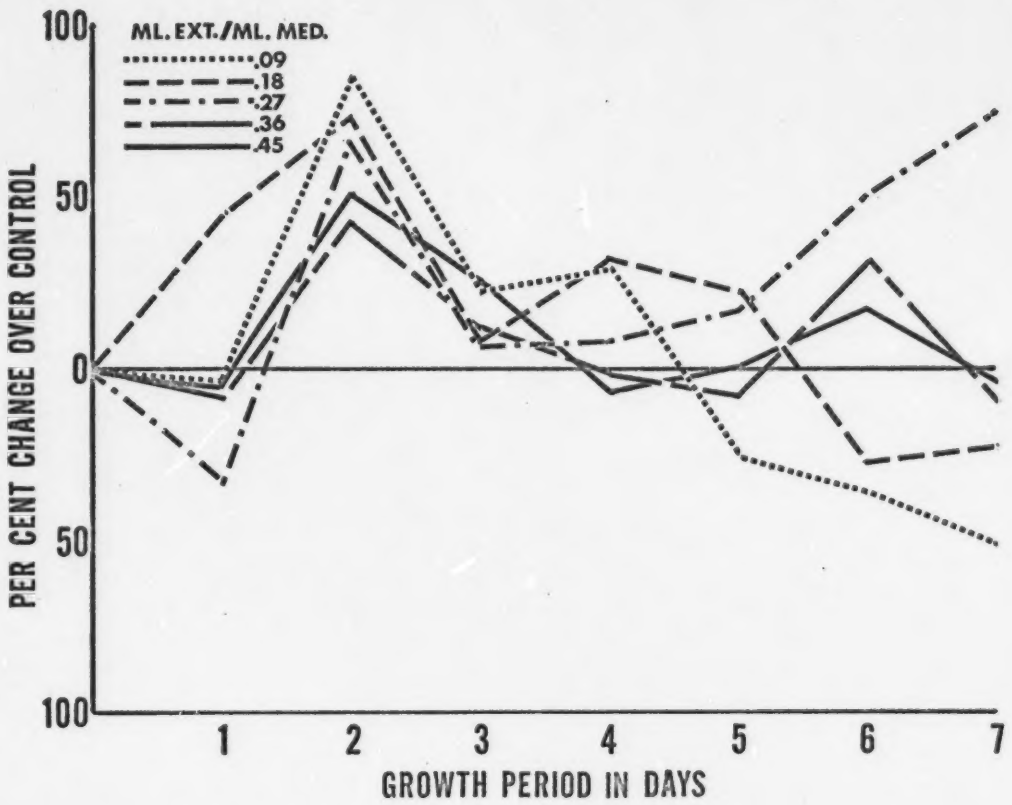

Fig. 10. Effect of different concentrations of Anabaena catenula extract on the growth of Trichomonas hominis during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

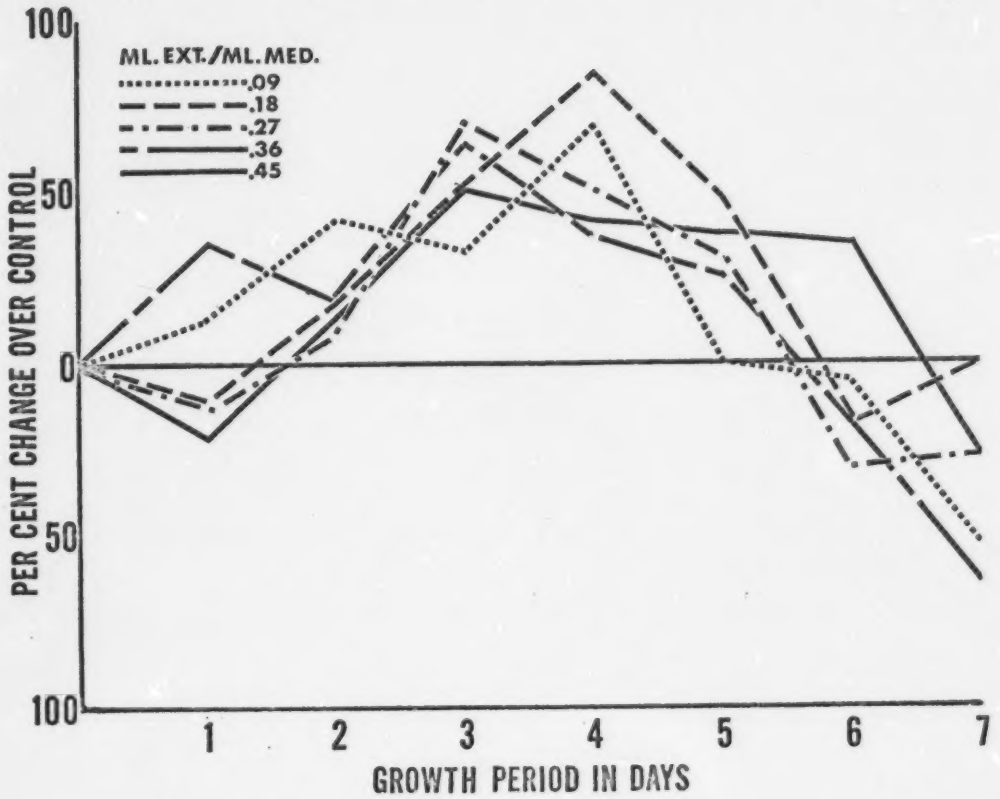

Fig. 11. Effect of different concentrations of Anabaena catenula extract on the growth of Trichomonas muris during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

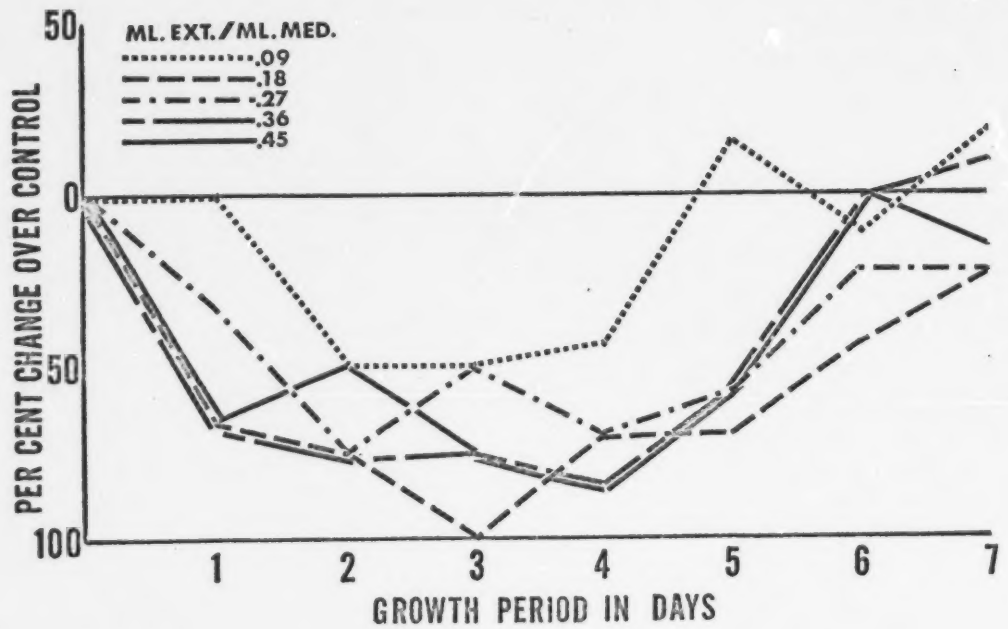

Fig. 12. Effect of different concentrations of Anabaena catenula extract on the growth of Trichomonas augusta during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

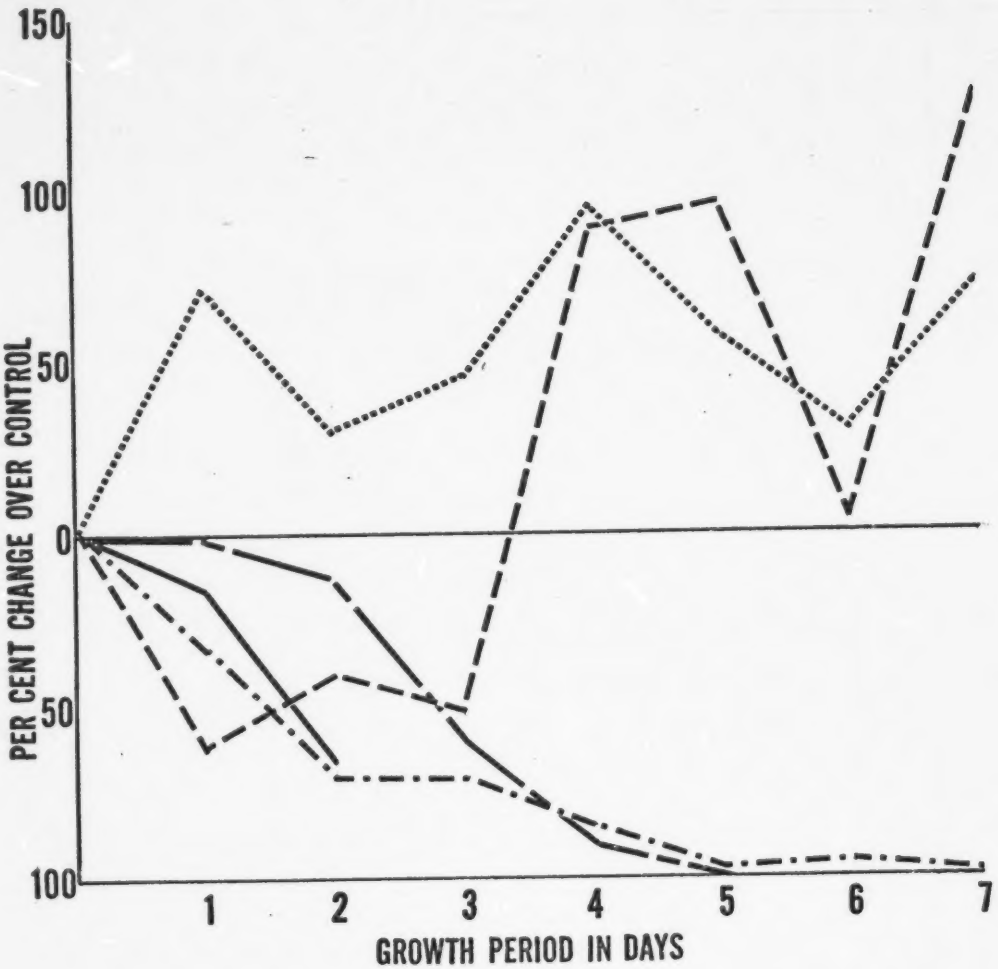

Fig. 13. Effect of different concentrations of Lyngbya sp. extract on the growth of Trichomonas muris during an incubatic period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

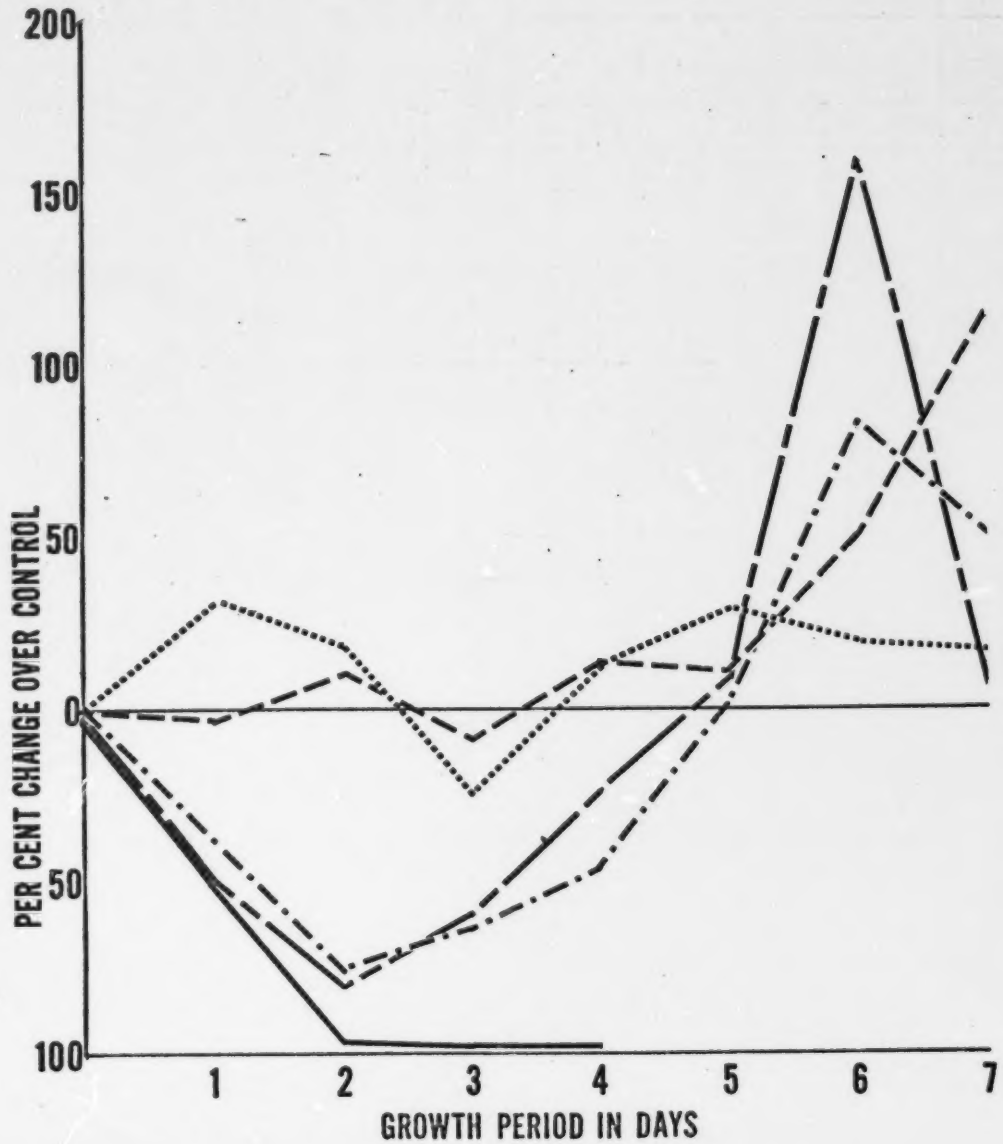

Fig. 14. Effect of different concentrations of Lyngbya sp. extract on the growth of Trichomonas hominis during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

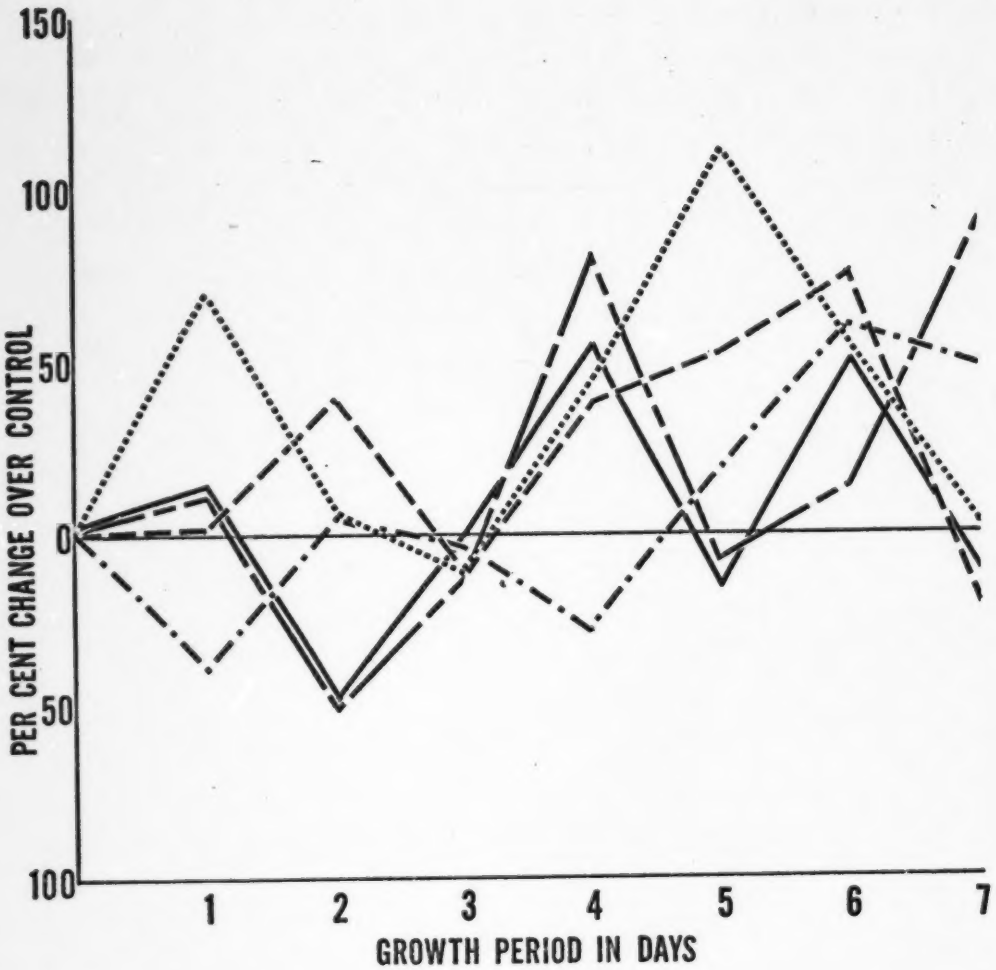

Fig. 15. Effect of different concentrations of Tolypothrix distorta extract on the growth of Trichomonas hominis during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

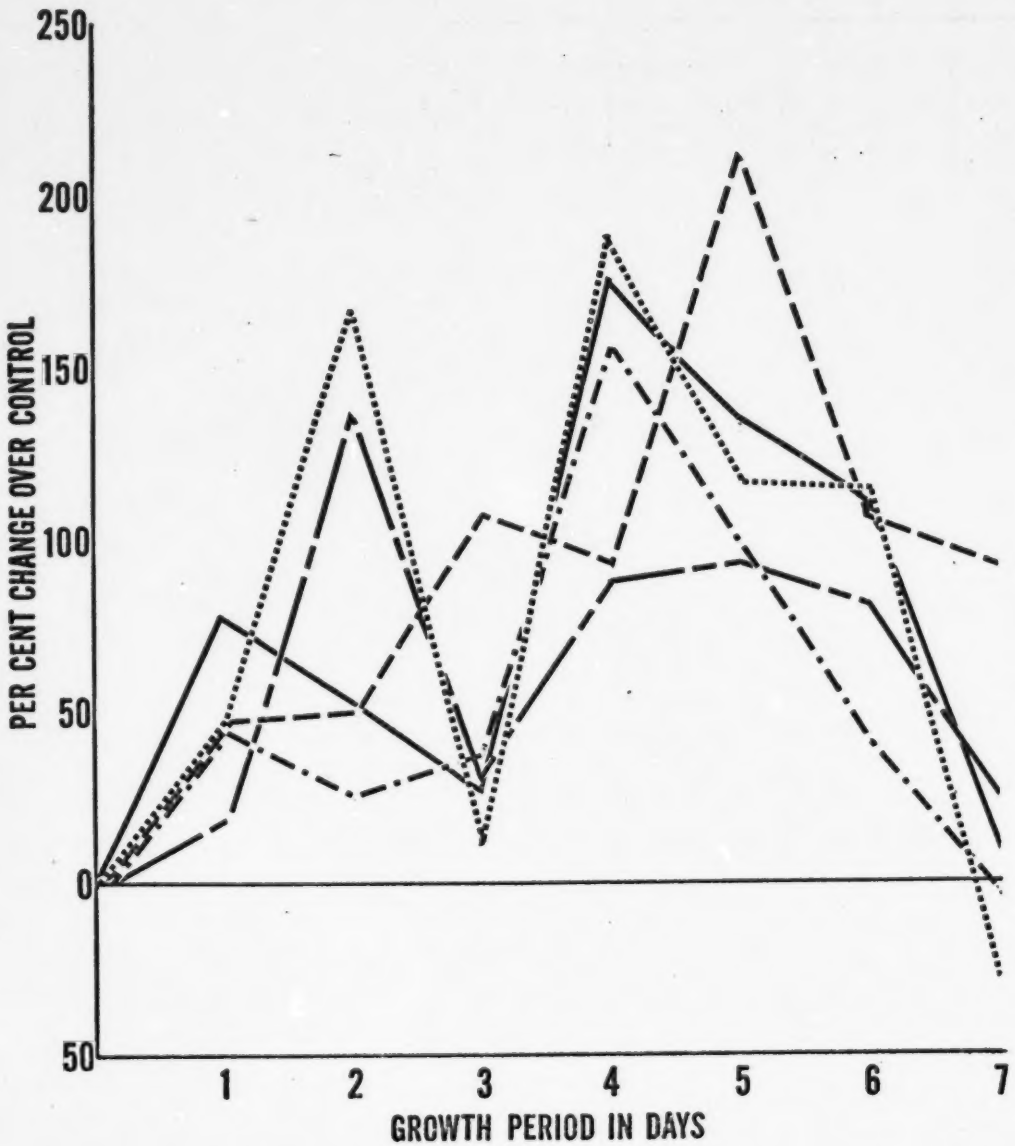

Fig. 16. Effect of different concentrations of Tolypothrix distorta extract on the growth of Trichomonas muris during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

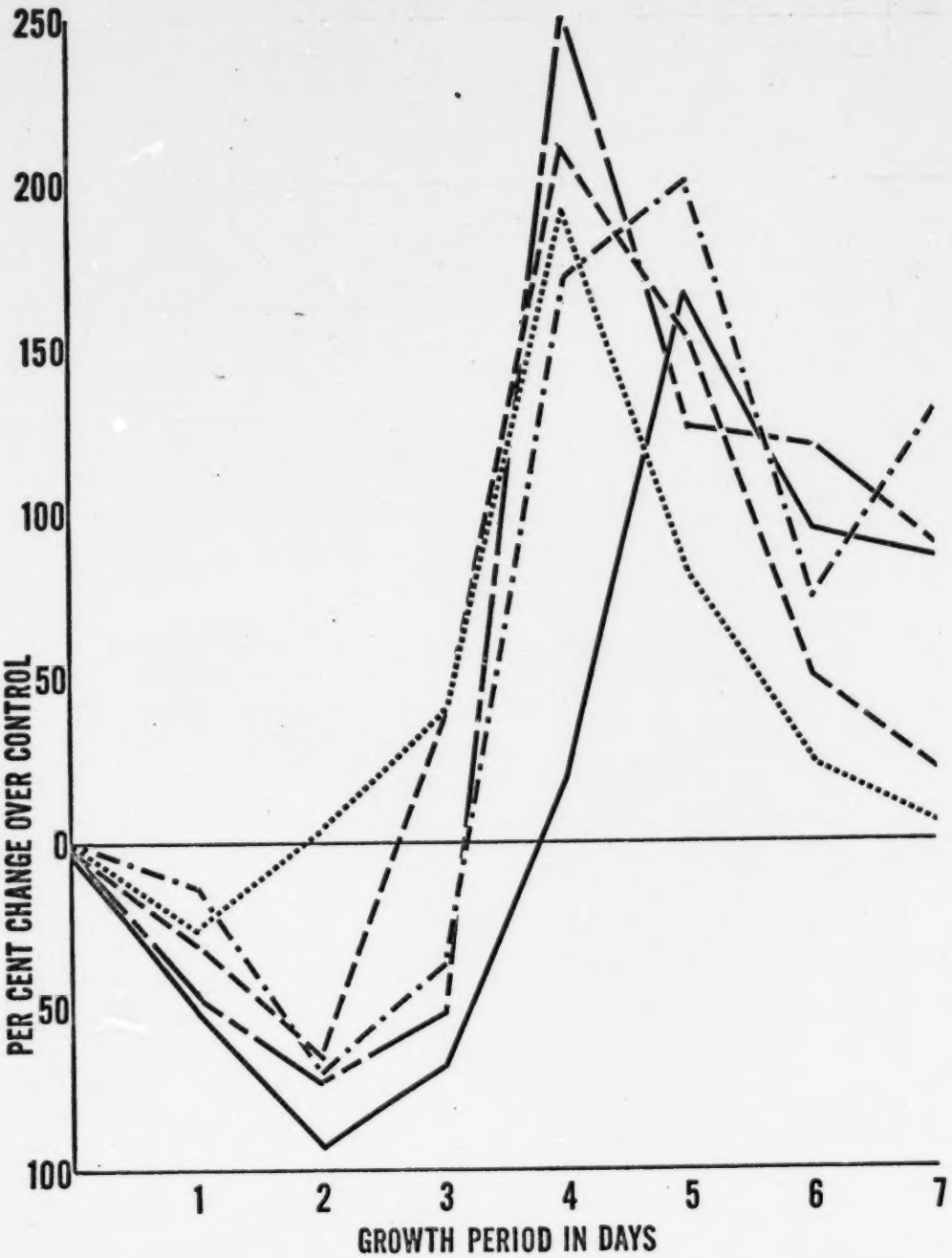

Fig. 17. Effect of different concentrations of Tolypothrix distorta extract on the growth of Trichomonas augusta during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

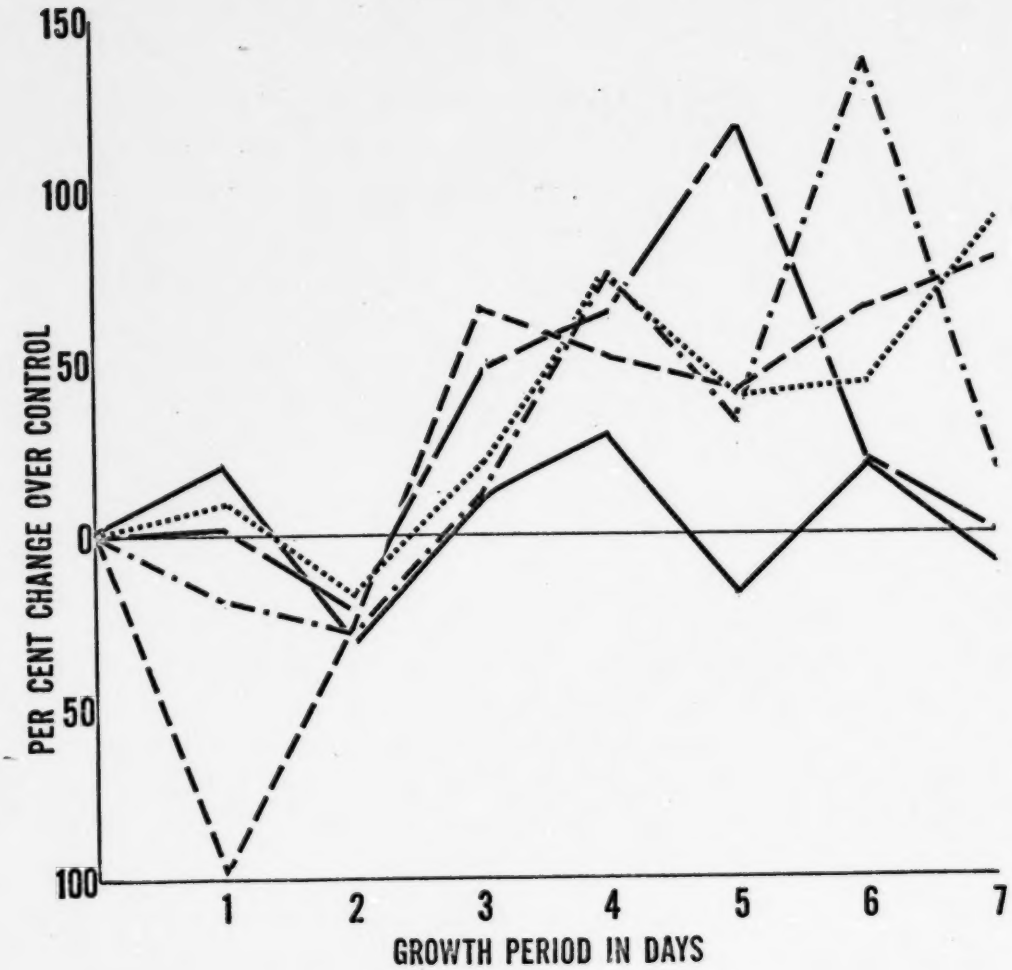

Fig. 18. Effect of different concentrations of Nostoc calcicola extract on the growth of Trichomonas hominis during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

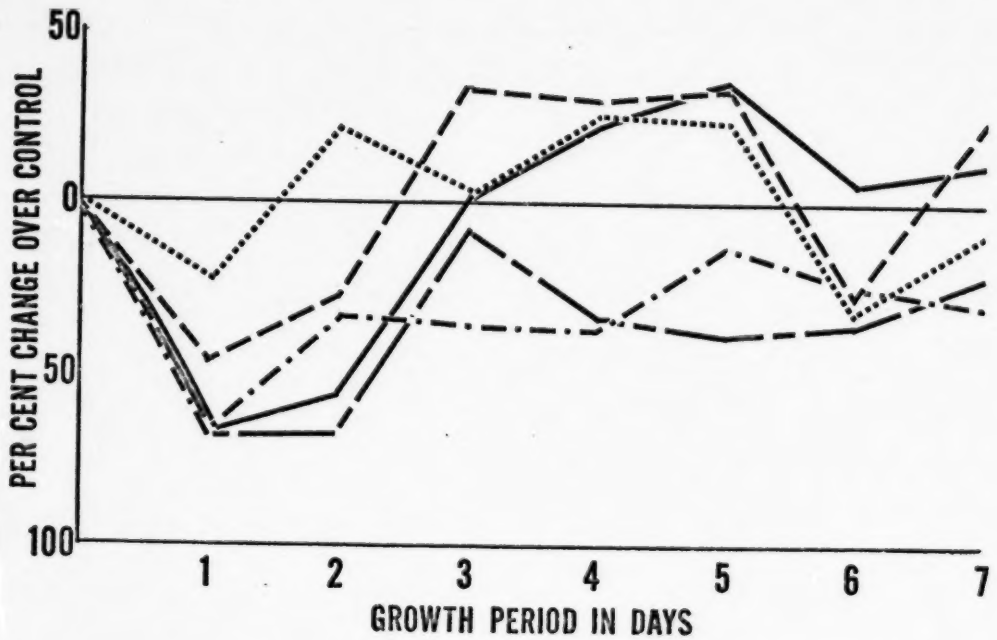

Fig. 19. Effect of different concentrations of Nostoc calciccla extract on the growth of Trichomonas muris during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

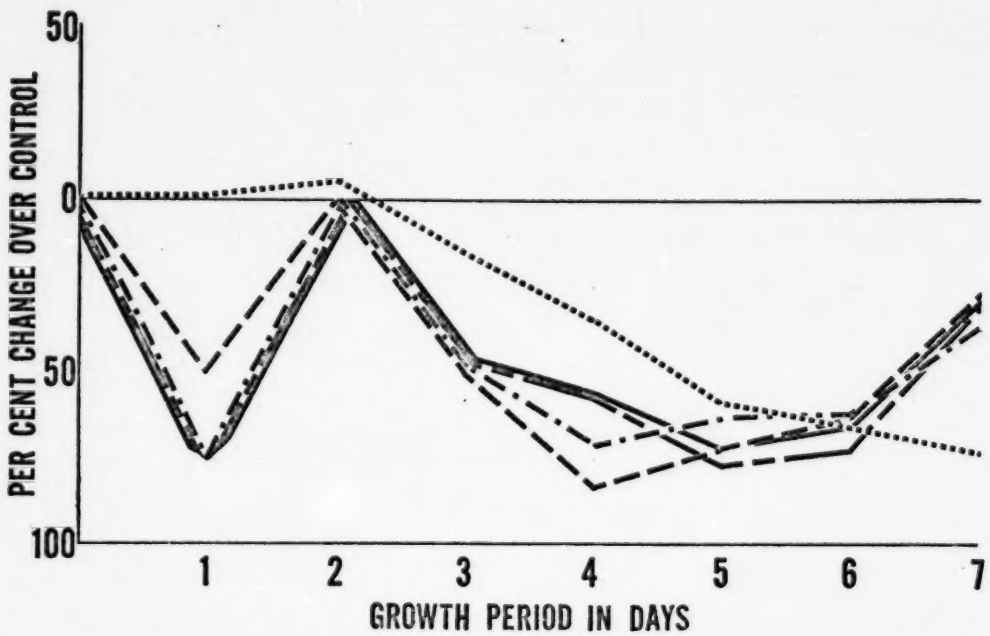

Fig. 20. Effect of different concentrations of Nostoc calcicola extract on the growth of Trichomonas augusta during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

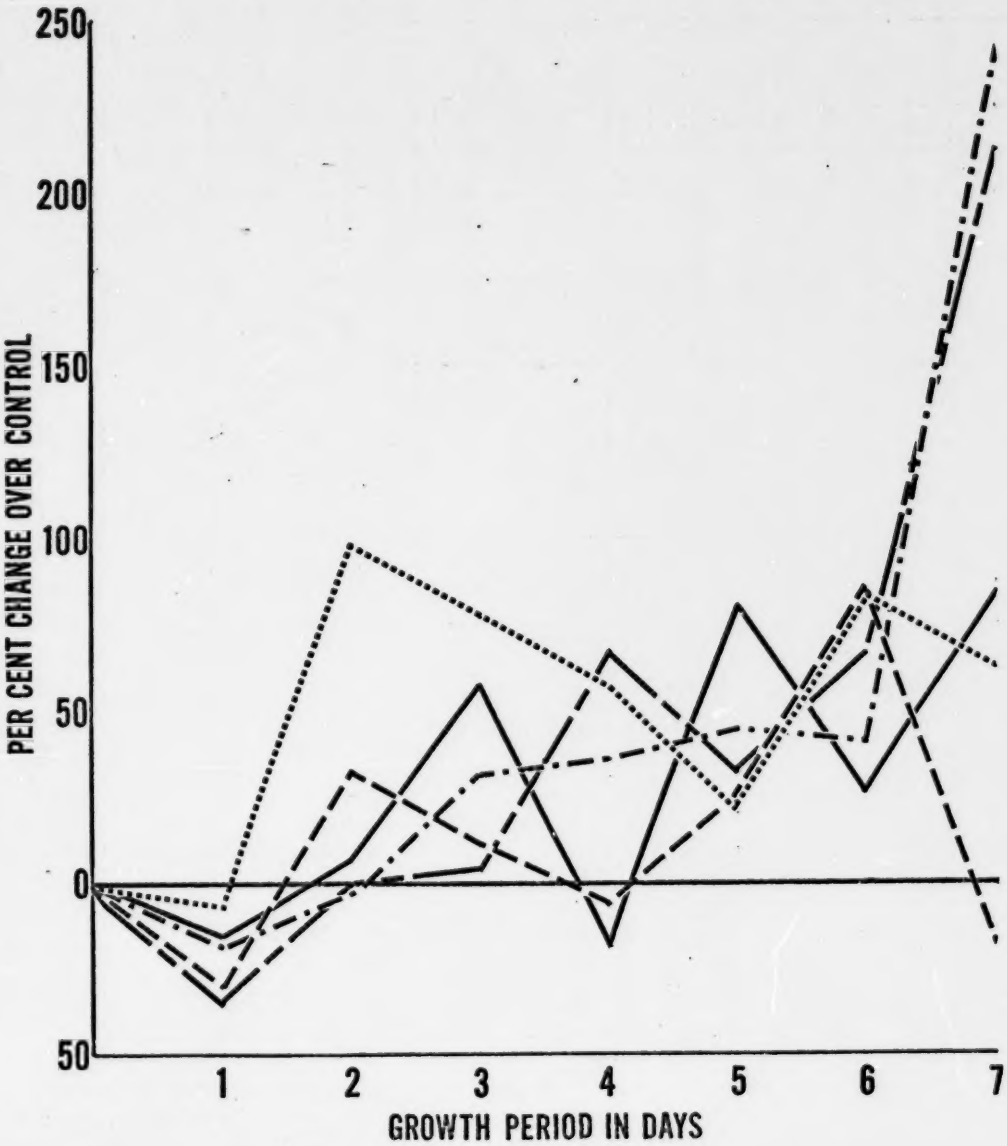

Fig. 21. Effect of different concentrations of Phormidium faveolarum extract on the growth of Trichomonas muris during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.

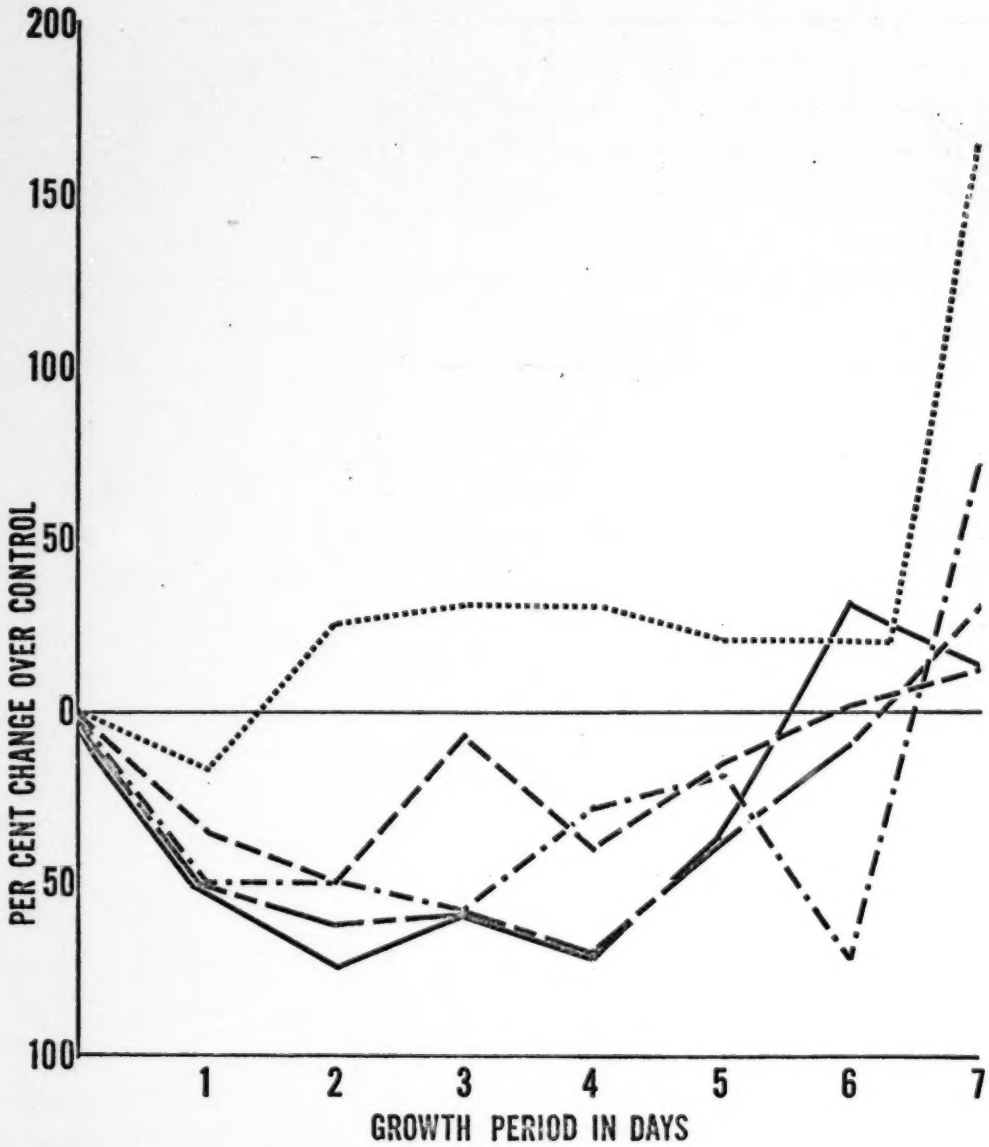

Fig. 22. Effect of different concentrations of Phormidium faveolarum extract on the growth of Trichomonas augusta during an incubation period of 7 days. Results are expressed in percentage change in the number of organisms per ml. medium in extract-treated cultures over the number of organisms per ml. medium in the control.